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Marine Research
1961 No. 5

Histological Changes in the Gonad associated with the Reproductive Cycle of the Herring

(Clupea harengus L.)

By A. B. BOWERS, B.Sc. and F. G. T. HOLLIDAY, B.Sc.

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(Clupea harengus L.*)*

By A. B. BOWERS, B.Sc.
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November, 1960

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Histological Changes in the Gonad associated with the Reproductive Cycle of the Herring *(Clupea harengus* L.)

INTRODUCTION

The assessment of sexual maturity has been for some time a valuable part of the routine sampling erring populations. Gonads are classified into arbitrary maturity stages by rapid visual assessment ch criteria as the shape and colour of the gonad, its size in relation to that of the body cavity, size gs, "freeness" of milt, etc. The classification of Hjort (1910) was (with slight modifications) recommded for general adoption by the Conseil International pour l'Exploration de la Mer (Ehrenbaum,). A more precise formulation of it was given by Lovern and Wood (1937). Jensen (1950) discussed relative merits of the International Scale and that proposed by Johannsen (1919) which was used while by Danish investigators. Naumov (1956) discussed another classification in relation to fecundity ovarian development.

The maturity assessments made in the routine examination of herring samples are fairly consistent in general quite adequate for obtaining an overall picture of the maturity range of a given sample erring. One defect of the published schemes is an inadequate description of the difference between gin gonad maturing for the first time, International Scale (I)II, and a gonad re-maturing after ning, International Scale (VII)II. Many workers consider that such a distinction can reliably be e and modifications of published maturity scales are used within laboratories, each with its own enclature. Such scales are readily convertible to the International (Hjort) Scale, which is set out w and which will be used throughout this paper.

The International (Hjort) Scale of Maturity Stages of the Gonad

Stage I: Virgin individuals: very small sexual organs close under vertebral column; ovaries wine-coloured, torpedo-shaped, about 2 to 3 cm long and 2 to 3 mm thick, eggs invisible to naked eye; testes whitish or greyish-brown, knife-shaped, 2 to 3 cm long and 2 to 3 mm broad.

Stage II: Maturing virgins or recovering spents: ovaries somewhat longer than half the length of ventral cavity, about 1 cm diameter, eggs small but visible to naked eye; milt whitish, somewhat bloodshot, of same size as ovaries, but still thin and knife-shaped.

Stage III: Sexual organs more swollen, occupying about half of ventral cavity.

Stage IV: Ovaries and testes occupying almost two thirds of ventral cavity; eggs not transparent, milt whitish, swollen.

Stage V: Sexual organs filling ventral cavity; ovaries with some large transparent eggs; milt white, not yet running.

Stage VI: Roe and milt running (spawning).

Stage VII: Spents: ovaries slack with residual eggs; testes baggy, bloodshot.
Some workers include recovering spents in a stage VIII.

The general gonad morphology is therefore well known. Descriptions may be found in Hjort (1910) Bullough (1940). Williamson (1945) describes in a series of articles the general morphology and also gross micro-anatomical structure of the herring gonad at particular maturity stages.

There is, however, a need for a detailed description of the histology of the herring gonad, throug its development, to determine the relationships between the maturity stage assessments and the r ductive cycle and also the range of gonad organization that is included in each stage. Such a descri would also provide basic information for such subjects as fecundity studies and rates of develop It is also necessary to provide a standard with which to compare the gonad state of herring broug maturity under controlled laboratory conditions, where purely morphological assessments are sufficient to detect modifications of the normal maturity cycle.

MATERIAL AND METHODS

Gonads were collected from fish of a wide range of size and age, and were placed in the mat groups of Hjort. The fish came from the Scottish east and west coasts, the Manx area and the D Bank. Fixation was in Bouin, Zenker, Flemming-without-acetic and formol saline. The material embedded in hard paraffin wax or ester wax. For some of the more advanced stages of the female g the blocked tissue was soaked in a 9:1 mixture of 60% alcohol and glycerine (Baker, 1950). This pr made sectioning of the yolk-laden eggs much easier.

Sections were cut at from 5 to 15 μ and stained with Heidenhain's azan stain, Mallory's triple and haematoxylin and eosin.

THE TESTIS

Stage I: Organisation of the Immature Testis

The immature virgin testis is a slender, elongated body of connective tissue in which lie the g cells; the tubulate structure of the mature testis (Cunningham, 1896) is not apparent at this sta thin sheet of peritoneum encloses a layer of fibrous connective tissue from which projections r through the interior of the testis forming a loose meshwork (Plate 1). The connective tissue fibre long; their nuclei are small, ovoid or spindle-shaped, with chromatin in the form of reticulate th which take up nuclear stains readily; one or more very small, deep-staining nucleoli are present. W the meshwork are numerous large, spherical, primary germ-cells, lying singly or in small groups, us within an envelope of non-fibrous connective tissue cells (Plate 2).

Solitary germ-cells may be up to 15 μ in diameter with a nucleus 10 μ in diameter; germ-ce groups are smaller. Large, solitary, primary germ-cells take up stains very sparingly; germ-cells in g have a slightly greater capacity for nuclear stains but otherwise look like the solitary cells. The chro in each case is typically in the form of numerous fragments dispersed at the periphery of the nu The single, large nucleolus, which lies in a clear, central zone of the nucleus, is joined to the peri by thin threads. Stages in nuclear division may be seen in solitary and in grouped germ-cells and thought that the primary germ-cells pass through several generations, becoming smaller at each di but retaining their characteristic appearance.

The connective tissue cells which invest the germ-cells vary widely in size, are flattened in side and roughly ovoid or irregular in surface view. Chromatin is scattered in small spherules throu the nucleus, and there may be one or more small nucleoli. The nuclear membrane is thin but tak acidic stains and shows clearly in prepared sections. The cytoplasm is granular and may possess acid inclusions. Some of the cells appear to be vacuolated (this may result from a loss of cell contents d fixation and dehydration). Similar cells occur in the general connective tissue of the testis where the frequently amoeboid in appearance. The cells described probably undergo cyclic changes. Those c associated with the germ-cells in the testis possibly function as Sertoli cells and are concerned wit nourishment of the germ-cells, but further study of the connective tissue complex is required. cells associated with the connective tissue include interstitial cells very similar to those described by and Hoar (1954), and haemopoietic cells (Marcuzzi, 1949).

As division of the primary germ-cells continues, the groups of cells so formed coalesce. At the same ıe the fibrous connective tissue becomes organised around the germ-cells into tubules, at first short l blind, but later interconnecting. The connective tissue cells which formed the envelopes around ups of germ-cells now line the tubules; the amoeboid appearance of some of these cells at this stage gests that they actively migrate from their original positions. Similar cells migrate along the connective ue fibres, and as the tubules increase in size the lining cells increase in number, partly by division l partly by recruitment from the wall of the testis.

At this stage the germ-cells nearest the tubule walls divide to form spermatogonia, and lumina ear in the centre of the tubules (Plates 3 and 4). The primary spermatogonia have a more compact leus than the germ-cells, and take up nuclear stains readily. The nucleus, which is 6 μ in diameter, ains the peripheral arrangement of chromatin and the central nucleolus described for the primary m-cells; there is little cytoplasm. The testis can probably remain in a resting condition following the duction of spermatogonia; cells more advanced than primary spermatogonia have not been seen estes classified as stage I.

In early stage I testes the only organised blood supply consists of a pair of unbranched blood vessels ning through the testis; extra-vascular corpuscles are common and widely distributed throughout loose connective tissue. On the formation of the tubules the blood becomes largely confined between tubule walls, and an organised vascular system is rapidly developed so that each tubule has an ciated blood supply.

ages II to V: Maturation of the Germ-cells

Developing virgin testes classified as stage II differ greatly in appearance from those described above; intermediate stages are seen and it appears that development, once initiated, proceeds rapidly. The ll of the testis, the tunica, is clearly defined as a layer of fibrous connective tissue 15 to 25 μ thick; it hicker on the inner face of the testis than on the outer. The blood system is highly organised, with ny small vessels lying in the tunica and connective tissue and capillaries associated with each tubule. bule formation is completed, and the sperm duct, which itself acts as a generative tubule, is defined ly as a tube running almost the whole length of the testis on the inner face near the dorsal edge. The ules form a plexus and are so arranged that they are more easily seen in longitudinal section than transverse section (Plate 5). At stage II the tubules are roughly 150 μ in diameter and their walls generally 4 to 5 μ thick; as their contents increase in volume the tubules become wider, and their lls thinner, presumably because they are stretched. The connective tissue border cells or lining cells viously described lie close to the tubule walls throughout the maturation cycle; at stage II they form ontinuous lining but by stage V they are spaced less closely along the walls.

Within the tubules are many groups of different kinds of cells, representing all stages of spermatogenesis luding tailed sperm (Plate 6). Groups of primary germ-cells are common in early developing testes become progressively less common as development advances. Primary and secondary spermatogonia ur in large numbers near the tubule walls; their nuclei may be dividing but division stages are not mmon in sections, and so presumably are of short duration. The number of spermatogonial divisions not been determined but the arrangement and number of cells suggest multiple division. Secondary rmatogonia have dense nuclei, 4 to 6 μ in diameter; their cell outlines are difficult to see. The earlier erations resemble primary spermatogonia but those formed by later divisions are difficult to distinguish m spermatocytes. The nuclear organisation of spermatocytes differs between groups of these cells not within the same group; in some the chromatin appears as a complex pattern of tangled threads l in others it is concentrated at the periphery of the nucleus or at one side. The changing appearance the spermatocyte is considered to be associated with maturation; different phases are essentially ilar to those of spermatocytes of the perch, described by Turner (1919), and the mudminnow *Umbra* (Kirtland), described by Foley (1926). They are most easily recognised when the chromatin is col- ed to one side of the nucleus at synizesis; the nucleus is then 3 μ in diameter.

Secondary spermatocytes are smaller than primary spermatocytes, the nucleus being about 2 μ in meter. The chromatin is usually dispersed along threads throughout the nucleus, but may be disposed

eccentrically in some groups of these cells. Even dispersal of chromatin is more common and this s probably represents a resting phase. No nucleolus has been observed in secondary spermatocytes.

Spermatids may be recognised by their small size (approximately 1 μ) and their concentrated ch matin which makes the nucleus look solid. They are initially spherical but later become slightly inden at one point so that they look kidney-shaped. This indentation is retained in the sperm when the is formed. The tail is long (approximately 20 μ).

At this stage the spermatozoa lie only in the centre of the tubules (Plate 5), and spermatids usu occur near clumps of sperm, but there is no clear serial arrangement of cells in order of maturity; cells nearest the tubule walls may be spermatocytes, spermatogonia or primary germ-cells.

In testes classified as stage III all the types of cells described above are found within the tubu but their total number has increased and the relative numbers of each type have changed: there many more spermatozoa, spermatids and spermatocytes. The tubules are wider than at stage II their walls are thinner.

The microscopic appearance of testes classified as stage IV may vary considerably; at early st IV primary and secondary spermatocytes are common within the tubules but at late stage IV they h been almost completely replaced by spermatids and spermatozoa. The production of spermatoc appears to stop in early stage IV so that their number becomes progressively less as they form sperma and sperm. Primary germ-cells and primary spermatogonia are found throughout the whole of maturity cycle but their development must be arrested at stage IV, since no new spermatocytes are form and they remain in a resting state, lying singly or in small groups close to the tubule walls, until onset of the next spawning season.

Spermatozoa become more numerous in the tubules as the testis matures until at stage V the tub are tightly packed with sperm and it is difficult to see the tails in sections. No spermatocytes or sperma can be seen. The tubule walls are extremely thin (2 to 3 μ in places) and the tunica is only 7 to 12 μ th The smallest tubules are by now 100 μ in diameter and the largest (excluding the main tube wh forms the sperm duct) are 0·5 mm.

Stage VI: The Running Testis

At stage VI the microscopic appearance of the testis changes greatly, the most obvious change be the absence of sperm from some of the tubules. The walls of empty tubules are thickened and may convoluted; those of tubules still full of sperm are thin and smooth, while partially emptied tubules sh some thickening and distortion. There is no doubt that the thickening of the tubule walls is due to c traction of the connective tissue fibres. Connective tissue border cells may be seen within the walls, primary germ-cells and primary spermatogonia in the folds of the walls.

Large, irregularly shaped cells with granular cytoplasm are seen lying free in the lumina of tub from which the sperm has been expelled (Plate 7). The cells vary in size but are usually about 28 μ al their greatest axis. In many of them there is a prominent, spherical or ovoid inclusion which stains dee with eosin; it appears to be suspended within a vacuole by fine fibrils of cytoplasm. No nucleus can seen in many of the cells but in others a faint-staining nucleus, 7 μ in diameter and slightly irregula outline apparently lies near the surface of the cytoplasm. The chromatin is typically in the form of v fine fibrils, with thickenings at intervals along them, dispersed throughout the nucleus; there is a fa prominent nucleolus. The origin and function of these cells is not known but it is suggested that they the "interstitial cells" seen earlier in a different phase; the nuclear structure, overall size and granu nature of the cytoplasm are similar in both.

Stages VII and (VII)II: The Spent Testis and its Recovery

In newly spent testes the fibrous connective tissue is much thickened by contraction and the wh organ is shrunken. The tunica is thick and on the outward-facing wall of the testis it is thrown i deep folds which run longitudinally. The tubules are distorted and have partially collapsed so t their walls are close together; there are usually some relict sperm and some extra-vascular blood c

les within the tubules. Some cells thought to be mainly spermatogonia and connective tissue cells ear to be breaking down spontaneously, and cell debris is common in the lumina of the tubules. nary germ-cells and primary spermatogonia, of typical appearance and presumably healthy, are ciated with the tubule walls.

Recovery is preceded by further contraction; in a young fish the testis becomes very compact and easily be mistaken macroscopically for a virgin (stage I) testis. The folds in the tunica, which is now o 100 μ thick, are closely pressed together and difficult to detect with the naked eye. Blood vessels ch had become dilated during maturation are greatly reduced in diameter; blood corpuscles are ined to the vessels and capillaries and can no longer be seen in the tubules. In normal testes relict m disappear from the tubules; it is thought that they are ingested by phagocytic cells derived from tubule walls. One or two specimens have been observed in which relict sperm had persisted for ral months; in these testes the tubule lining cells were arranged in a complete border next to the us connective tissue of the tubule walls. In most testes these cells lose their orderly arrangement are found lying free in the lumina of the tubules (the lumina are much reduced in size but rarely ided by the contraction of the testis).

As recovery proceeds the tubules become lined with connective tissue cells, and groups of dividing n-cells and primary spermatogonia become prominent (Plate 8). At first these groups are dispersed g the tubule walls and contain few cells; their number increases by cell division until they fill the les three or four months before spawning. The tubules at this stage are still narrow (20 to 60 μ in ieter) and their walls unstretched. The recovering testis can be distinguished microscopically from arly developing virgin testis at this time by the thick folded tunica, by the well developed connective e of the tubule walls and by the extensive blood system. Shortly after this stage secondary sperma- nia and, a little later, primary spermatocytes appear in the tubules, the testis increases in volume, unica and tubule walls extend to accommodate the increased contents, with the result that, at this , it is impossible to distinguish microscopically between developing virgin testes at stage II and vered spents redeveloping.

SITES IN THE TESTIS

number of testes contained cysts of *Eimeria sardinae* Thelohan (Schäperclaus, 1954). The cysts lay n the tubules of the testis, embedded in the germinal cells, at all stages of maturity from II to VI. age VI the parasites were found among the ripe sperm in the sperm duct, and sections through the of the herring showed that they were passed out to the exterior with the sperm. A few cysts remained e tubules at stage VII.

SSION

has been shown that Hjort's maturity stage I covers the organisation of the connective tissue of estis and the differentiation of spermatogonia from the primary germ-cells. Stages II (virgin) and epresent a phase of rapid multiplication of cells with the production of secondary spermatogonia, natocytes, spermatids and sperms. There is no marked qualitative difference between stages II II, but so long as stage II covers both virgins and recovering spents the distinction between stages d III remains a practical necessity for general fisheries work. At stage IV production of spermato- ceases, and spermatogonia then present are carried through to the following spawning season. V represents the completion of maturation of the germ-cells available for the current season's ning. A major change occurs in the testis at stage VI with the shedding of ripe sperm and consequent action and distortion of the tubule walls. There is evidence from observation of spawning in tanks iday, 1958) and from histological examination of the region of the genital pore that discharge of n is under muscular control; the absence of muscle fibres in the testis itself suggests that the force red for emitting the sperm arises from the contraction of elastic tissue, the control being exercised at enital opening. This implies that the stored sperm are under pressure while they are retained in stis.

t stage VII the tubule walls are contracted, thickened and distorted, and the tubule lining cells

are cast off; there are relict sperm and extra-vascular blood corpuscles within the tubules. Some nective tissue cells and some germinal cells undergo degeneration and resorption. Stage II (recove is long in time, and represents a gradual change from the newly spent state up to the time tha earliest formed sperm is present in the reorganised tubules prior to a new spawning season; most o time appears to be taken up with the division of primary germ-cells and formation of primary sperm gonia.

The correspondence between Hjort's maturity scale and the presence of different cells of the gerr line is shown diagrammatically in Fig. 1, together with a rough time-table applicable to Autumn spaw herring.

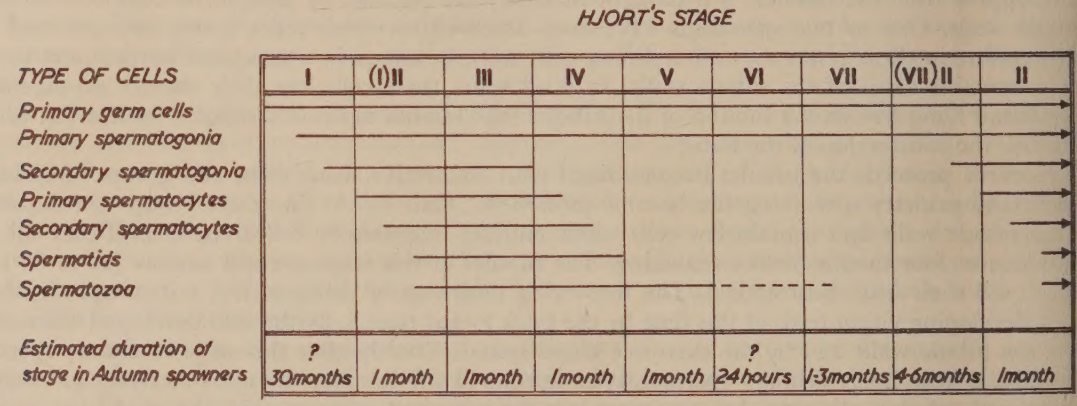


Fig. 1. Germinal cells present at the stages of the Hjort maturity scale. (Autumn spawning herring)

It was found that some testes which were diagnosed histologically as recovering spents had classified as virgin stage I or stage II by experienced fishery workers. The criteria of size and c laid down in maturity scales in general use are inadequate for distinguishing between early develc gonads and some gonads which are recovering, particularly those of young fish. Use of a hand lens a fine needle to determine whether or not the testis wall is folded is helpful when the gonads to be c fied are less than half the length of the body cavity, but difficulty still exists when folds in the tunic tightly pressed together.

Several authors have described primary germ-cells similar to those in the herring testis, and observed them in a resting condition at all states of the testis (e.g. Bullough, 1939, in *Phoxinus;* W 1943, in *Salmo gairdneri*); James (1946) and Matthews (1938) reported spermatogonia in the tes *Lepomis* and *Fundulus* at all states of maturity. In the herring both primary germ-cells and pri spermatogonia are found at all times of the year. The course of recovery of the spent herring testis indi that division of primary cells that had remained quiescent during the previous spawning season rise to the new crop of spermatogonia and subsequent derivatives. No evidence was obtained of migr of germ-cells into the maturing virgin testis or the recovering spent testis of the herring like that desc by Turner (1919) in the perch, or of migration of germ-cells within the testis (Geiser, 1924; Gol

Abbreviations used in Plates

pg,	primary germ-cells;	bv,	blood vessels;	pth,	primary theca;
sg,	spermatogonia;	bc,	blood cells;	ch,	chorion;
sc1,	primary spermatocytes;	p,	peritoneum;	o,	oocyte;
sc2,	secondary spermatocytes;	t,	tunica;	do,	developing oocyte;
s,	spermatozoa;	sd,	sperm duct;	ro,	resting oocyte;
c,	connective tissue cells;	v,	vacuolated cells;	th.e.,	theca externa;
f.	fibrous connective tissue;	od,	oviduct;	od.ep.,	oviducal epithelium;
		g,	granulosa cells;	y,	yolk.

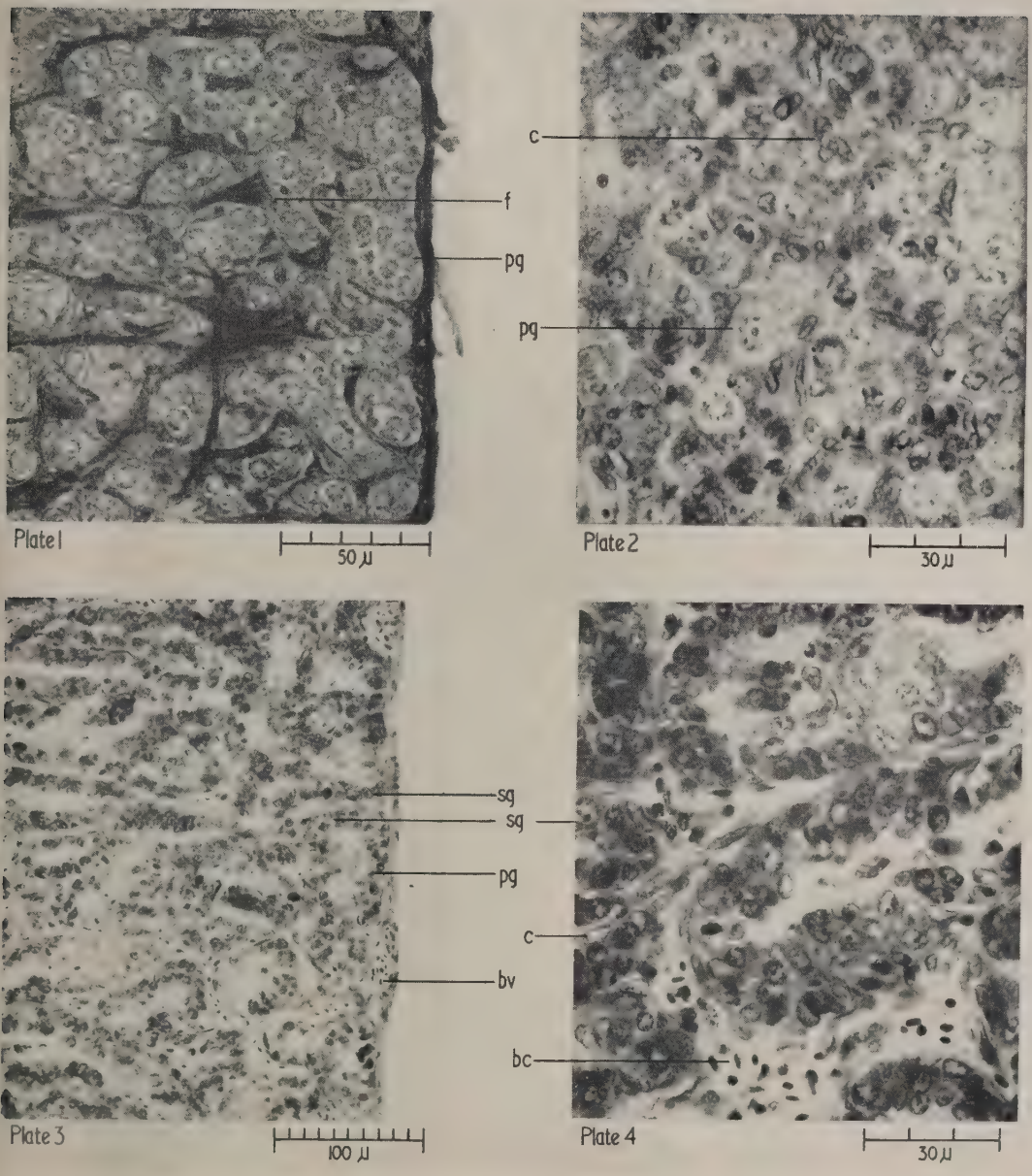
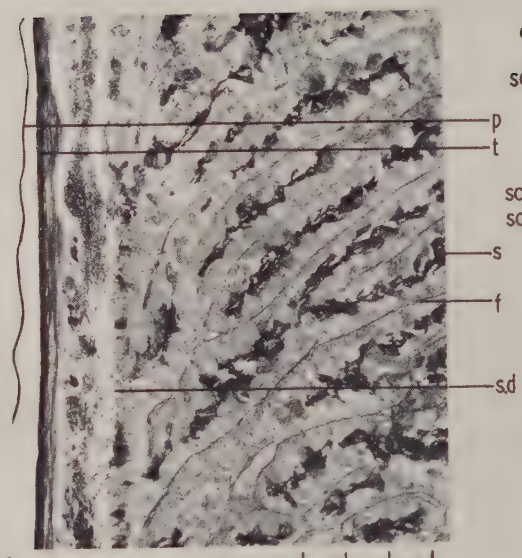
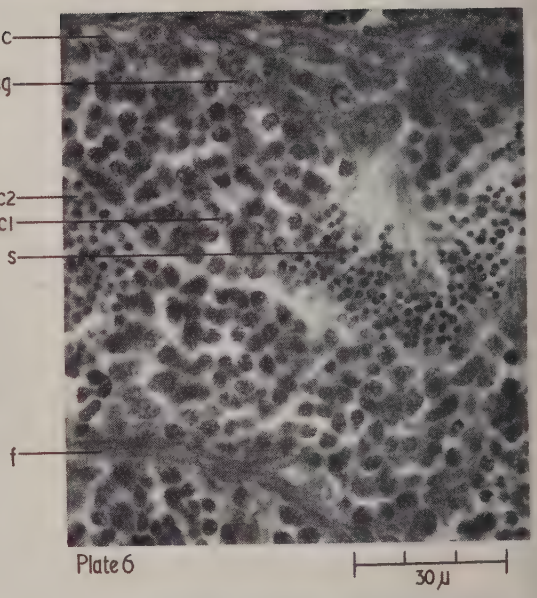


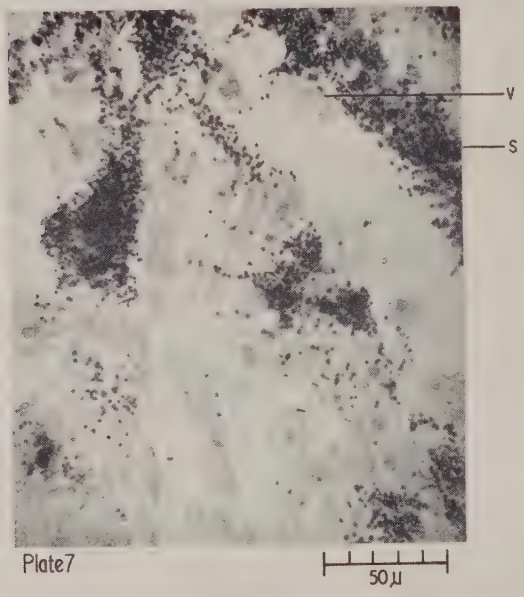
PLATE 1. Immature virgin testis showing primary germ-cells and fibrous connective tissue. (azan)

PLATE 2. Immature virgin testis showing primary germ-cells and cells of the connective tissue envelope. (haematoxylin and eosin)

PLATES 3 and 4. Immature virgin testis at stage of tubule formation, showing pale-staining primary germ-cells, darker-staining spermatogonia, connective tissue lining cells and blood vessels. (haematoxylin and eosin)







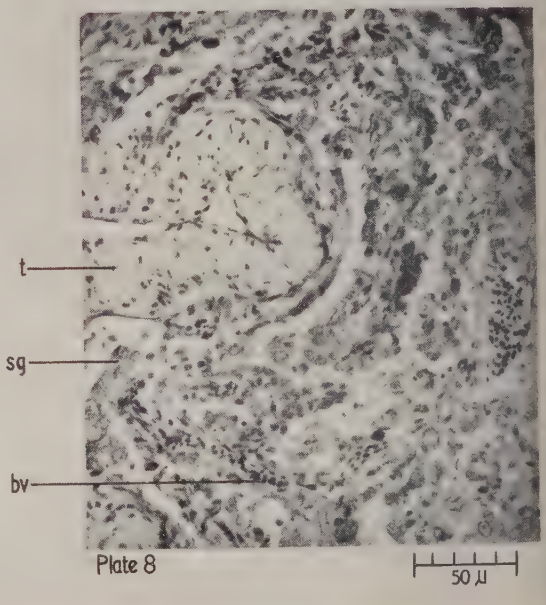


PLATE 5. Maturing testis showing tubules opening into the sperm duct. The black masses in the centre of the tubules are spermatozoa. The peritoneum has partly stripped off the tunica (this is a post-mortem change). (azan)

PLATE 6. Maturing testis showing types of cells. (haematoxylin and eosin)

PLATE 7. Running testis showing vacuolated cells. (haematoxylin and eosin)

PLATE 8. Recovering spent testis showing deeply folded tunica, and spermatogonia lying along the connective tissue. (haematoxylin and eosin)

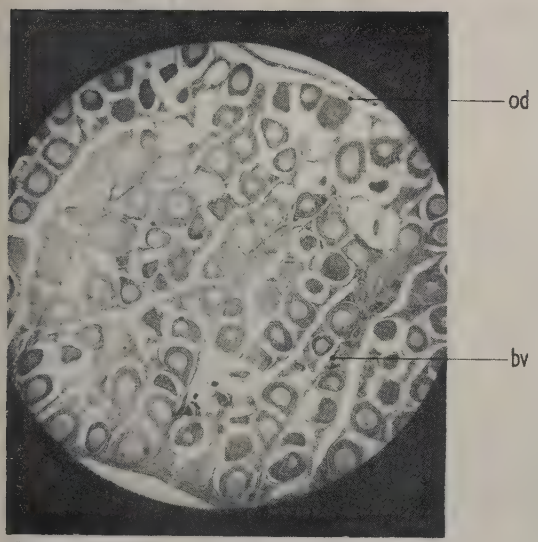


Plate 9 300 μ

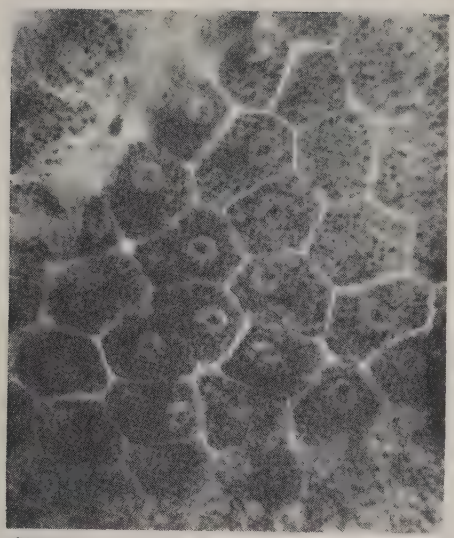


Plate 10 10 μ

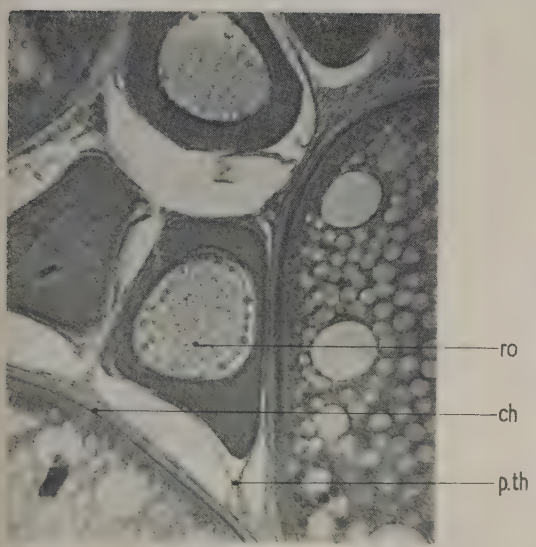


Plate 11 50 μ

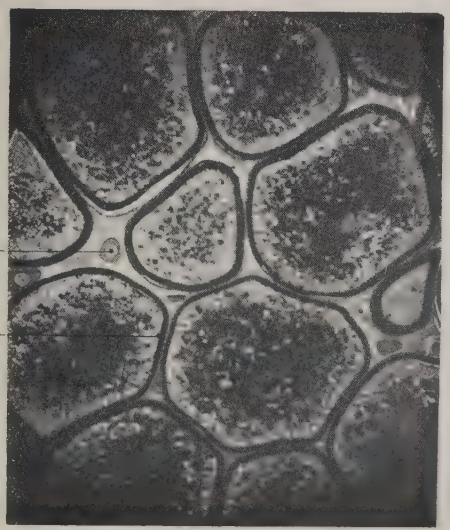


Plate 12 300 μ

PLATE 9. Stage I. Immature virgin ovary showing oocytes arranged along blood vessels. (haematoxylin and eosin)

PLATE 10. Surface view of the granulosa cells of the follicle. (azan)

PLATE 11. Stage II. Early stages of yolk formation and resting oocytes. (haematoxylin and eosin)

PLATE 12. Stage IV. Developing and resting oocytes, yolk formation well advanced and chorion formed. (azan)

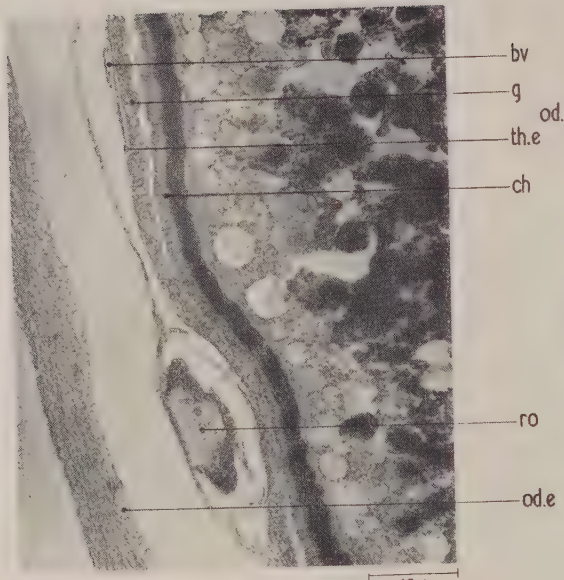


Plate 13

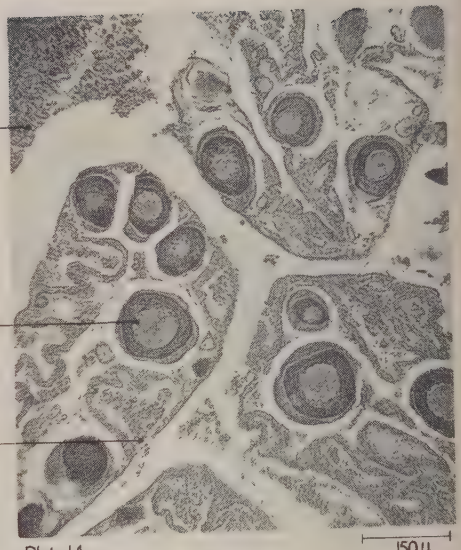


Plate 14

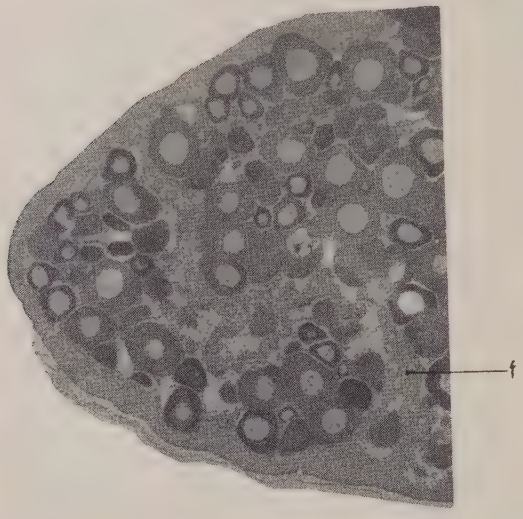


Plate 15

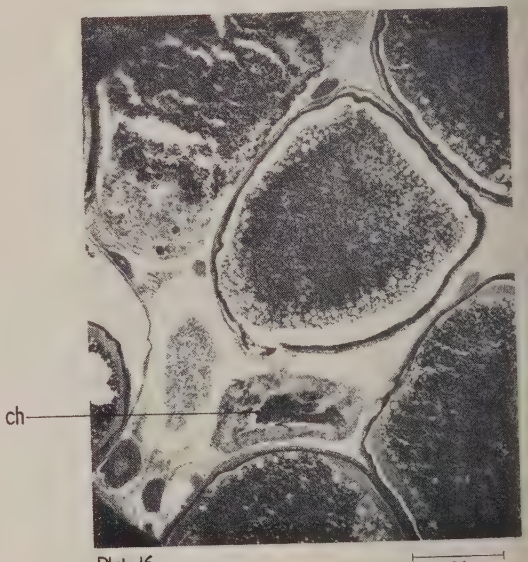


Plate 16

PLATE 13. Stage IV. The structure of the follicle and chorion. (azan)

PLATE 14. Stage VII. Empty follicles, with granulosa cells being cast off. (haematoxylin and eosin)

PLATE 15. Stage VII-II. Thick connective tissue and renewed complements of oocytes. (haematoxylin and eosin)

PLATE 16. Stage IV. Pre-ovulatory degeneration of eggs. (azan)

7), or of transformation of connective tissue cells into germ-cells. The immature testis of the halflin year old) herring contains primary germ-cells, and it is believed that all subsequent sex cells are ved from them by division which gives rise either to more primary germ-cells, some of which will ain quiescent for a long period, or to primary spermatogonia.

The tubule-lining cells in the herring testis are similar in some respects to the migrating germ-cells cribed and figured by Turner (1919). In the ripening testis they are disposed along the tubule walls manner very like that indicated for migrating germ-cells in Turner's Figure 29, but the history of lining cells and the absence of any stages which could be regarded as transformation into definitive m-cells suggests that they are not sex cells. The presence of lipoids in the lining cells is indicated by r reaction with Sudan black dye, and in this respect they resemble the lobule boundary cell described some teleosts by Marshall and Lofts (1956) and regarded by them as Leydig cell homologues, but her histochemical tests will be necessary before the function of the lining cells can be determined sfactorily.

THE OVARY

AGE I: THE IMMATURE OVARY

The ovary is covered with a thin sheet of peritoneum, containing scattered small nuclei. Immediately neath this lies the ovary wall, the tunica, a connective tissue structure 5 to 30 μ thick. A large number incomplete connective tissue septa are present throughout the ovary, within which run the branches the ovarian blood vessels; the germ-cells lie in rows within the septa on either side of the blood vessels ate 9). The irregularly shaped free edges of the septa project into the oviduct, which forms the dorsal rt of the ovary.

e germ-cells and oogonia

Within the septa of the immature ovary are cells of four characteristic types. Groups of large primary m-cells, with a nuclear diameter of 8 to 10 μ and faintly staining cytoplasm, are surrounded by smaller nnective tissue cells whose spindle-shaped nuclei are 4 to 9 μ in length and contain deeply-staining cleoli and scattered chromatin. These cells resemble strongly those described and pictured in the le gonad.

The oogonia derived from the primary germ-cells are large circular cells, 10 to 14 μ in diameter, th a nuclear diameter of 5 to 8 μ. The nucleus contains one central nucleolus, linked to the periphery the nucleus by chromatin threads.

e oocytes

The transformation of the oogonium into the primary oocyte is accompanied by a general growth both nucleus and cytoplasm. The smallest oocytes found had a cellular diameter of 10 to 14 μ and a clear diameter of 7 to 8 μ. There is a rearrangement of the nuclear contents; the nucleoli become ouped around the periphery and the general chromatin structure distributed in the centre.

e follicle

The growth of the oocytes within the connective tissue septa results in their projection into the lumen the ovary. A thin layer of connective tissue is still retained around them and forms the primary theca late 11). This is the theca externa of the future follicle. Within the connective tissue are the blood ssels which will supply the follicle, passing along the narrow neck of connective tissue which now links e primary theca to the septum. Each oocyte has an individual blood supply.

As the oocytes grow, a proportion of them become surrounded by a single layer of small cells, similar if not identical with the connective tissue cells associated with the primary germ-cells. These cells grate through the strand of connective tissue, assuming a spindle shape, and become arranged in a

single-layered envelope surrounding the oocyte, closely applied to the theca externa. They are at f small, flattened and elongate and possess large nuclei. They rapidly reach their maximum size, the length being from 10 to 15 μ with a nuclear diameter of 4 to 8 μ, and in surface view are polygo (Plate 10). These cells form the innermost layer of the follicle—the granulosa layer. The cytoplasm the fully differentiated cells contains many strongly acidophil granules; the nucleus contains a num of nucleoli situated centrally. The follicle in which the egg develops consists then of an envelope of ce the granulosa, enclosed externally by a thin sheet of vascular connective tissue, the theca externa (Plate 1

Stage II: Yolk Formation

Once the granulosa cells have migrated around the oocyte several changes occur within it. T cytoplasm stains less densely and the nucleus more so. Yolk vesicles appear in the cytoplasm, at f peripherally but later spreading inwards. At this stage of development there are two types of ooc within the ovary, one acquiring yolk, the other without yolk (Plate 11). Both types of oocyte have reac a size of about 110 μ, with a nuclear diameter of 70 μ; at this size, however, the oocytes with y (the maturing oocytes) have the granulosa layer of the follicle while those without yolk (resting oocytes) have no granulosa layer, although the primary theca is present. The resting ooc remain at this stage throughout the subsequent ovarian cycle. Such oocytes show no signs of degenerat and it may be assumed that the blood vessels of the primary theca maintain them in this stage. Ther always a greater proportion of residual oocytes than maturing oocytes. The complete development the oocytes depends, therefore, on the properties of the granulosa cells. The acquisition of yolk is dep dent on these cells and so also is the formation of the chorion (the thickened outer membranes of oocyte) which occurs at this stage.

The chorion

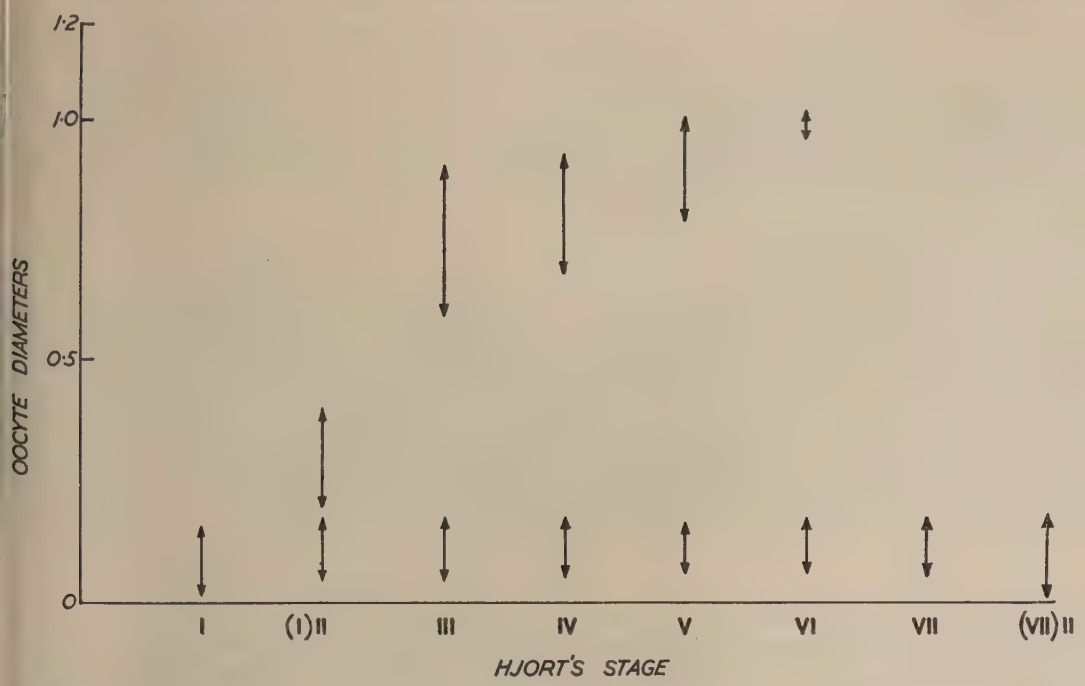
When fully formed the chorion is a triple-layered structure, shown in Plate 13. The first sign of formation is an increase in density of the peripheral cytoplasm of the oocyte (Plate 11). The outer la of the chorion is the first to be differentiated. It appears as a thin layer, coarsely striated (proba porous) in a radial plane and, when carefully differentiated, stains blue with azan. Very soon after the second and third layers are formed within it. These are more finely striated in the radial plane a stain bright red with azan. There is then a thickening of all three layers.

The nucleus of the egg is at this stage still a large structure, situated in the centre of the cell. Surro ding it is a layer of dense cytoplasm free of yolk; the peripheral cytoplasm has now many yolk vesic Outside the chorion is the fully formed follicle. The eggs are now visible to the naked eye when ovary is viewed macroscopically.

Stages III to V: The Phase of Growth

The gonads in stages III to V of the Hjort scale come to occupy more of the body cavity. This incre in size is seen microscopically to be a result of growth of the individual eggs, and not of any incre in the number maturing. There is an increasing difference in size between the resting and matu oocytes (Plate 12 and Fig. 2).

All parts of the cytoplasm now gain yolk, and as far as can be seen histologically there is a defi sequence in its acquisition. The first yolk gained is very soluble in alcohol, the vesicles staining b with azan, and it occupies the peripheral zone of the cytoplasm. During the later maturation this y type is still present but is confined to the periphery. A second type of yolk, less soluble in alcohol staining bright red with azan is also present in the periphery, but mainly concentrated in the cen regions. At the latest maturity stages, i.e. just prior to and at ovulation, it becomes more sensitive to orange-G component of azan. The blue staining component is no longer present. The nutritive mater transported from the blood vessels by the granulosa cells, must pass through the three-layered chor The brightly staining granules found in the granulosa cells are similar in staining reaction and in gen appearance to the secondary yolk of the egg, and it seems possible that this is yolk being transfe from the blood vessels of the theca externa to the egg.



2. Range of oocyte sizes present at the stages of the Hjort maturity scale. (Scottish east coast autumn spawners)

The yolk droplets in the oocyte remain discrete throughout the maturation process. In some fish, whiting and Norway pout (Gokhale, 1957), just prior to ovulation the yolk droplets become dis-ed throughout the cytoplasm, which is then homogeneous. Just prior to ovulation in the herring yolk droplets increase in size, apparently by fusion. This is also the situation in the Pacific herring, *ea pallasii* (Yamamoto, 1956).

As the egg increases in size the follicle surrounding it enlarges. The granulosa cells increase in number the theca externa is augmented from the original connective tissue septum.

It has been shown (Baxter, 1958) that egg size at maturity varies in herring from different regions spawning at different times of the year. The absolute measurements of egg diameters of the different ks are not, therefore, relevant to this general description of maturation, although Fig. 2 shows the ge of oocyte sizes found in samples of Scottish east coast herring. It may be said that when released n the ovary the diameter of the egg varies from 1·1 mm in the Scottish east coast spawners to 1·7 mm he Norwegian spring spawners. It would seem that, although growth in absolute terms differs, the tive growth and maturation of the oocytes follows the same general pattern in all herring.

GE VI: OVULATION

The release of the egg from its follicle (ovulation) marks the last phase in the development. Immediately r to ovulation there is an increase in the vascularity of the ovary, which can be seen macroscopically. this stage the follicles burst, both theca externa and granulosa splitting at a point opposite to the k strand, and the eggs are released into the lumen of the ovary. All eggs appear to be released from follicles simultaneously.

The lumen of the ovary is continuous with a wide duct in the dorsal portion of the ovary (Plate 9). s duct later forms the oviduct, which unites with that from the other ovary, and a common oviduct es to the exterior. The lining of the ovarian portion of the duct is composed of cells which up to this e, i.e. just prior to ovulation, have been small, cubical in shape, with a cell height of from 5 to 8 μ

(Plate 13). These cells at ovulation increase in height to 25 to 40 μ (Plate 14), become club-sha and commence secretion into the lumen of the ovary. The eggs are released into this secretion and t pass via the oviduct to the exterior.

The nature of the secretion is not known, but is seems likely to serve either or both of the functi of maintaining the eggs after ovulation and forming the adhesive substance which serves to attach eggs to the substrate and to each other at spawning.

Stage VII: The Spent Ovary

Immediately after ovulation the empty follicles, connective tissue and blood vessels are forced the eggs in the expanded lumen to the periphery of the ovary. The ruptured follicle, which was o as big as the egg, now contracts, probably under the influence of elastic fibres in the theca exter This contraction results in an apparent thickening of the layer of granulosa cells as they become fol and condensed. The theca externa itself also thickens, and following this the granulosa cells are cast passing via the lumen of the ovary to the oviduct and then presumably to the exterior (Plate 14), leav the connective tissue theca externa as a calyx (Bretschneider and Duyvené de Wit, 1947). The rupt of the follicle wall apparently does not result in the breaking of blood vessels; no extra-vascular corpus were found.

Stage (VII)II: The Recovering Ovary

The residual, small oocytes which did not develop are still present (Plate 14) and are augmen in number by the development of further oocytes. Throughout stages VII and early (VII)II prim germ-cells are abundant in the ovary and at stage (VII)II these become oogonia and oocytes.

The development of the new oocytes and the consolidation of the once stretched connective tis results in a decrease in volume and general thickening of the whole ovary (Plate 15). At this stage ovary differs from that in the immature fish by the presence of much more connective tissue—the thicke remains of the thecae externae. The ovary wall itself is thickened (up to 300 μ) and often folded (Plate this provides the best macroscopic criterion for identifying the recovering spent gonad. The subsequ cycle of growth and maturation re-stretches the connective tissue and it is difficult, if not impossi to tell that the developing ovary has passed through the spent phase. The maturing of the gonad subsequent spawnings follows a pattern identical with the first; a proportion of the oocytes matu and a greater proportion remains in the resting phase.

Post-ovulatory Degeneration of Eggs

After the herring has spawned there may be a few residual eggs left in the oviduct or lumen of ovary. In some cases, commonly called "egg bound", one or both of the oviducts may be blocked ar large proportion of ovulated eggs remain unshed. It is not uncommon to find one gonad spent and other full of ovulated but unshed eggs. The residual eggs break down and are resorbed in the ovid The process appears to be one of autolysis and not one of phagocytosis.

Pre-ovulatory Degeneration of Eggs

This is not an extensive phenomenon in the herring, though occasionally in the spent fish some appear not to have ovulated. In a few fish caught at stages IV and V, about one-tenth of the gonad sho signs of regression and in all instances the eggs had reached an advanced stage of development with chorion fully formed and the presence of abundant yolk. The onset of breakdown is marked by disappearance of the yolk from the peripheral regions of the egg. This is followed by degeneratio the egg; the chorion breaks down in a number of places and portions of it sink into the general yolk m This latter is presumably absorbed by the ovary and the oocyte shrinks until all that remains is a of connective tissue containing the distorted and shrunken chorion (Plate 16).

CUSSION

It is interesting to examine Hjort's maturity assessments in relation to the histology of the ovary. general stage I includes the initial growth phases of the maturing virgin to the point at which the yte becomes surrounded by the follicle cells. At late stage I and early stage II the maturing oocytes differentiated from the resting oocytes. At stage II the follicle is fully formed and at this stage yolk ıcquired and all three layers of the chorion differentiated. Recovering spents are also classified as ge II. These have been found to include (a) fish with ovaries in which the granulosa cells are in the cess of being cast off from the thickened connective tissue, with only the resting oocytes of the previous r present, and (b) recovered spents in which the number of oocytes has been augmented and in some es maturing and resting oocytes differentiated.

Thus stage II covers a number of different phases of gonad development and the addition of a separate ge (VII)II to include the recovering spents until such time as the full complement of oocytes has been ıblished would seem very desirable.

Stages III, IV and V correspond with the increase in size of the oocyte and the gaining of yolk. e secondary yolk is well established at stage III and the chorion fully formed and thickened.

At stage VI the general increase in vascularity occurs, the cells of the oviduct increase in height and ınmence secretion. Ovulation occurs and the large transparent eggs lie free of the follicles, tightly ked in the lumen of the ovary.

The burst follicles have contracted at stage VII and the granulosa cells become folded. At late stage I these cells start to be cast off. There is an increased amount of connective tissue, the ovary wall has ckened and folded and a further crop of oocytes is formed.

On several occasions gonads that had been identified as maturity stage I proved on sectioning to be overing spents. Although the gonads were small and apparently immature they showed the typical ck connective tissue of the spent fish and occasionally the remains of cast-off follicle cells.

The fecundity of the herring is apparent very early in development, at stage II when the follicles formed. The resting oocytes which are apparent at stages (I)II and (VII)II most probably form the turing oocytes of the subsequent year. This conclusion was also reached by Naumov (1956) in a study the Murmansk herring. Estimates of the ratios of resting to maturing oocytes in sections of the ovary re made and there is always a higher proportion of the resting type. This accords well with Baxter's 58) finding that in the herring fecundity increases with age. It appears that a "resting phase" of one ır is necessary before the oocyte is capable of further maturation. This means that the fecundity in one year is fixed at the immediate post-spawning stage of the previous year, and that any external tors, e.g. food supply, can have an influence then. Also the conditions existing during the first two ırs of the herring's life determine the fecundities of the first and second spawnings.

The development and fate of the follicle in the herring places it in the "aglandular" category of tschneider and Duyvené de Wit (1947). No glandular tissue is formed after ovulation nor does it ult from the pre-ovulatory degeneration of eggs. From the specimens of the latter examined so far s not clear whether the whole ovary is degenerating or only a proportion of the eggs. Pre-ovulatory generation of eggs has been described in many species. Andreu and dos Santos Pinto (1956) record n another clupeoid, the sardine, *Sardina pilchardus* Walbaum. In many species the degeneration of the is accompanied by a proliferation of the follicular cells. A so called glandular organ arises from the nulosa layer and is called by Bretschneider and Duyvené de Wit the pre-ovulatory corpus luteum. has been suggested (Hoar, 1955) that this organ forms the main endocrine tissue in the ovary and is sent in the majority of teleosts. In the herring, however, such degeneration does not appear to be an ensive phenomenon and the granulosa cells do not proliferate; in this species at least it cannot be arded as having an endocrine function.

GENERAL DISCUSSION

Several features are common to the development of both the testis and the ovary. The gametes a produced from primary germ-cells which are very similar in both. The connective tissue cells investi the germ-cells are also alike; in both the male and the female these cells eventually line the connecti tissue enclosing the gametes, the tubules in the male and the follicles in the female. A strong similar exists between the tubules and the follicles, both as regards structure and function.

There are, of course, a number of striking differences between the developmental processes in t male and female. The number of gametes produced from a primary germ-cell differs greatly betwe the testis and ovary. The sperm duct acts as a tubule, i.e. it is lined with generative cells, whereas t oviduct has a specialised epithelial lining. There is a lack of muscle fibres in the male gonad and there also a difference in the rate of maturation of the gametes. Mature sperm are present in the male gon at stage II; the eggs do not reach maturity until stage VI. The increase in size of the testis as matur advances is a result of the increasing number of germinal cells; this contrasts with the condition in t ovary where increase in size of the gonad results from increase in size of a fixed proportion of oocyt

In general the Hjort scale is adequate for fishery investigations; it is most difficult to apply when t gonad is in the spent condition and fish at stage (VII)II have been classified as stage I by experienc fishery workers. Stage II covers a whole range of gonad states, but it is difficult to subdivide it on maci scopic characters alone and the time factor in routine analysis of large numbers of fish prevents the u of more detailed techniques.

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SUMMARY

Gametogenesis in the herring is described in detail. The immature virgin testis contains prima germ-cells and primary spermatogonia lying within a body of connective tissue.

At the beginning of the first spawning season the connective tissue forms a complex of tubules whi open into the sperm duct. Gametogenesis proceeds within the tubules and the duct.

Some sperm are produced early in the maturity cycle; the quantity of sperm increases steadily the gonad matures. The tubule walls and the tunica of the testis become stretched and thin as the gon increases in volume.

The increase in size of the testis as maturity advances is a result of the increasing number of germi cells.

Just before spawning the testis is packed with sperm and there are some primary germ-cells and prima spermatogonia. At spawning the sperm are shed but the primary germ-cells and spermatogonia rema in the testis. The tunica and tubule walls contract, thicken and fold.

After spawning there is a long recovery period, during which spermatogonia are produced from t primary germ-cells remaining in the testis. Division of these cells produces a new crop of sperm t following spawning season.

No organised interstitial tissue was recognised in the herring testis; some cells of the connecti tissue resemble interstitial cells described by various authors.

The immature ovary contains primary germ-cells, oogonia and oocytes lying within connecti tissue septa which project into the lumen of the ovary.

During development the oocyte becomes enclosed within a follicle consisting of an outer layer of nective tissue and an inner layer of granulosa cells.

After the formation of the follicle, yolk is deposited and the chorion formed. At this stage the fecundity he fish is evident; resting oocytes are always more numerous than maturing oocytes. Increase in of the ovary during development is a result of increase in size of the individual oocytes and not of increase in the number developing.

Ovulation is brought about by the rupture of the follicles. All the eggs are ovulated together; at this e the epithelium of the oviduct becomes highly secretory.

After spawning the connective tissue in the gonad contracts and thickens and the granulosa cells cast off into the oviduct. A further crop of oocytes (i.e. for a third spawning) is then formed.

Pre- and post-ovulatory degeneration of eggs is described.

The Hjort system of classification of gonad maturity in the herring is related to the biological cycle. ssification based on macroscopic external criteria can be loosely correlated with internal microscopic nges in the gonad. Macroscopic criteria for distinguishing between recovering spent fish and im- ture virgin fish are unsatisfactory.

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